SCREENING TEST FOR SEMINAL FLUID

A. SCOPE

A.1 The acid phosphatase test is used as a screening test for semen. Naphthyl phosphate is acted upon by the acid phosphatase enzyme to produce naphthol, which then combines with the diazo blue B dye to give a violet-colored complex. Seminal acid phosphatase originates in the prostate gland. Although present in other body fluids, it occurs in seminal fluid at concentrations 20 to 400 times higher than that of other body fluids. It is also present in humans at higher concentrations when compared to other animal species.

While it is generally accepted that high concentrations of acid phosphatase are present in semen, a positive test does not confirm the presence of semen. Acid phosphatase occurs in other human tissues, animals, and plants including the following: human urine, human milk, human liver, human kidney, red blood cells, snake poisons, cauliflower, brussels sprouts, rice bran, clover, sweet almonds, lupines, malen blume, bind weed, lucerne seeds, corn cockle, bacteria, and mold fungi.

Since this assay is dependent upon the amount of enzyme present, a negative acid phosphatase test does not necessarily mean that a stain does not contain semen. It is possible to have semen present in such dilute amounts in a stain that a positive reaction is not obtained.

B. QUALITY CONTROL

B.1 Known positive and negative controls must be tested when a new lot of acid phosphatase reagents are prepared.

B.2 Results must be documented in the Laboratory Asset Management System (LAM).

B.3 A positive control must be tested each day of use prior to testing unknown or suspected semen samples.

B.4 Results of the day of use quality control testing must be documented in the case notes and include the lot#, expiration date and positive control tested.

B.5 If the used quality control measures do not produce the expected result, the reagents will not be used on evidentiary samples and troubleshooting will be performed. New solutions or materials may be required.

C. SAFETY

C.1 Treat all biological samples as potentially infectious. Gloves, a face mask, eye protection (e.g. safety glasses or a face shield) and a lab coat must be worn.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Distinguish all waste as general, biohazard or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 Make the Buffer

D.1.1 Prepare by mixing 1 ml of glacial acetic acid and 2 g sodium acetate in 100 ml deionized water.

D.2 Make Solution 1

D.2.1 Prepare by adding 0.13 g of alpha-naphthyl phosphate, disodium salt to 50 mL of buffer and mixing until dissolved.

D.3 Make Solution 2

D.3.1 Prepare by adding 0.25 g of Fast Blue B salt to 50 mL of buffer and mixing until dissolved.

D.3.2 For the combination of solution1 and solution 2, assign a lot# and expiration date (date of earliest expiring component or eight weeks, whichever is sooner) and document this in the Laboratory Asset Management System (LAM).

D.4 Solutions can be made up in bulk, aliquoted into test tubes, capped and frozen. When needed, one tube of each solution can be thawed for use. Solutions 1 and 2 are stable frozen for eight weeks. Once thawed, solutions should be disposed of either when the color of Solution 2 becomes significantly darkened or at the end of each day (whichever comes first). Depending on need, the above quantities may be increased or decreased to make a larger or smaller bulk batch.

D.5 A known semen stain is used to test the solutions.

E. EQUIPMENT

E.1 Glass beakers or flasks

E.2 Graduated cylinders

E.3 Balance

E.4 Stir plate and stir bars

E.5 Disposable plastic pipettes

E.6 Test tubes and caps

E.7 Cotton swabs

F. PROCEDURES

F.1 Add one drop of Solution 1 to the tip of a cotton swab and hold the swab firmly in contact with the suspected semen stain for 30 seconds and then remove. Typically, the approximate location where the swab contacted the stain on the item will be marked. Alternatively, cut a small (~1-2mm^2) piece of a suspected stain and place on a glass microscope slide or filter paper and add one drop of Solution 1.

F.2 Add one drop of Solution 2 to the swab or onto the cutting.

G. INTERPRETATION GUIDELINES

G.1 If there is an immediate purple color which starts to develop with the addition of solution 2, this is a positive result. If the purple color takes several seconds to develop and the color is less intense than the known standards, qualifiers will be added to the positive result in the notes and this result should be viewed cautiously. Note: Positive results can be obtained from vaginal secretions, bacteria, and certain vaginal sprays, douches, and contraceptives, but will typically be less intense and slower than with semen.

G.2 When there is no color change or only weak color develops after 1 minute this is a negative result.

G.3 The acid phosphatase test is a presumptive test only. When desired, confirmation of the presence of semen may be attempted by microscopic observation of spermatozoa. Additionally, the detection of the prostatic antigen protein p30 may be used as an indicator of the presence of seminal fluid.

H. REFERENCES

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